

Product Information

Anti-phospho-c-Abl [pTyr⁴¹²]

Developed in Rabbit, Affinity Isolated Antibody

Product Number **C 5240**

Product Description

Anti-phospho-Anti-c-Abl [pTyr⁴¹²] is developed in rabbit using a synthetic phosphorylated peptide derived from the region of c-Abl that contains tyrosine 412 as immunogen. There are two widely expressed forms of c-Abl produced by alternative splicing, known as 1a and 1b. The tyrosine 412 is the phosphorylation site of form 1b (the more commonly used form). The corresponding phosphorylation site in 1a is tyrosine 393. The antiserum is affinity purified using epitope-specific affinity chromatography. The antibody is preadsorbed to remove any reactivity toward a non-phosphorylated peptide. Anti-phospho-Anti-c-Abl [pTyr⁴¹²] specifically recognizes c-Abl phosphorylated at tyrosine 412 (approximately 140-150 kDa).

The antibody detects human c-Abl. Mouse c-Abl (100% homology) is expected to cross react. The antibody has been used in immunoblotting applications.

c-Abl is a 140-150 kDa non-receptor protein tyrosine kinase whose precise functions are not known, but it appears to play a role in growth factor and integrin signaling, cell cycle regulation, cytoskeletal reorganization, neurogenesis, and in responses to DNA damage and oxidative stress. c-Abl kinase activity is increased *in vivo* by diverse physiological stimuli including ionizing radiation, entry into S phase, integrin activation, and platelet-derived growth factor (PDGF) stimulation. c-Abl contains various protein binding domains that appear to enable it to regulate the functions of many proteins by forming complexes, most notably three isoforms of the oncogenic protein BCR/ABL.

c-Abl becomes fully activated by sequential phosphorylation of tyrosines 412 and 245.

Reagent

Anti-phospho-Anti-c-Abl (pTyr⁴¹²) is supplied as a solution in Dulbecco's phosphate buffered saline (without Mg²⁺ and Ca²⁺), pH 7.3, with 1.0 mg/ml BSA (IgG and protease free) and 0.05% sodium azide.

Precautions and Disclaimer

Due to the sodium azide content, a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazards and safe handling practices.

Storage/Stability

Store at -70 °C. Upon initial thawing freeze the solution in working aliquots for extended storage. Avoid repeated freezing and thawing to prevent denaturing the antibody. Do not store in frost-free freezers. Working dilution samples should be discarded if not used within 12 hours. The antibody is stable for at least 12 months when stored appropriately.

Product Profile

The supplied reagent is sufficient for 10 blots.

A recommended working concentration of 0.1 to 1.0 µg/mL is determined by immunoblotting using fibroblasts transfected with oncogenic delta SH3-Abl.

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working concentration by titration test.

Results

Peptide Competition

1. Fibroblasts transfected with λSH3-Abl were resolved by SDS-PAGE on a 10% polyacrylamide gel and transferred to PVDF.
2. Membranes were blocked with a 5% BSA-TBST buffer overnight at 4 °C.
3. After blocking, membranes were preincubated with different peptides as follow:

Lane 1	no peptide
Lane 2	non-phosphorylated peptide corresponding to the immunogen
Lane 3	a generic phosphotyrosine containing peptide
Lane 4	immunogen

4. After preincubation membranes were incubated with 0.50 µg/mL c-Abl [pTyr⁴¹²] antibody for two hours at room temperature in a 3% BSA-TBST buffer.
5. After washing, membranes were incubated with goat F(ab')₂ anti-rabbit IgG alkaline phosphatase and signals were detected.

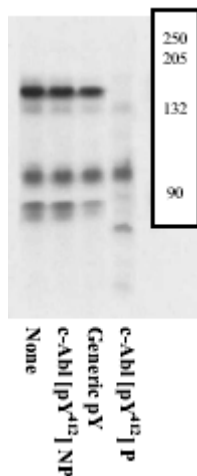


Figure 1 Peptide Competition

The data in Figure 1 show that only the peptide corresponding to c-Abl [pTyr⁴¹²] blocks the antibody signal, thereby demonstrating the specificity of the antibody.

References

1. Cong, F., et al. Interaction between UV-damaged DNA binding activity proteins and the c-Abl tyrosine kinase. *J. Biol. Chem.*, **277**, 34870-34878 (2002).
2. Furstoss, O., et al., c-Abl is an effector of Src for growth factor-induced c-myc expression and DNA synthesis. *EMBO J.*, **21**, 514-524 (2002).
3. Brasher, B.B. and R.A. Van Etten c-Abl has high intrinsic tyrosine kinase activity that is stimulated by mutation of the src homology 3 domain and by autophosphorylation at two distinct regulatory tyrosines. *J. Biol. Chem.*, **275**, 35631-35637 (2000).
4. Plattner, R., et al. c-Abl is activated by growth factors and Src family kinases and has a role in the cellular response to PDGF. *Genes Dev.* **13**, 2400-2411 (1999).

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